Molecular Probes of Gating and Open Channel Conformational Transitions of Mechanosensitive Ion Channels

Final Report

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Final Report

A. Statement of the Problem Studied

The objective of this project was to elucidate the molecular mechanisms which control the gating behavior and ion transport properties of mechanosensitive ion channels in skeletal muscle. The project utilized recordings of single-channel activity to address two general questions: 1) How does an absence of the cytoskeletal protein, dystrophin, influence the mechanosenitive gating of single channels? 2) What is the mechanism by which positively charged aminoglycoside antibiotics inhibit the flow of current through these channels?

B. Summary of the Results

Expression of mechanosensitive ion channels in developing muscle cells. These experiments examined the activity of single mechanosensitive ion channels in recordings from cell-attached patches on myoblasts, differentiated myotubes, and acutely isolated skeletal muscle fibers from wild type and *mdx* and *dy* mutant mice. The predominant form of channel activity recorded with physiological saline in the patch electrode arose from a ~25 pS mechanosensitive ion channel. Channel activity was similar in undifferentiated myoblasts isolated from all three strains of mice. By contrast, channel activity in *mdx* myotubes was ~3-4 greater than in either wild type or *dy* myotubes and arose from a novel mode of mechanosensitive gating. Single mechanosensitive channels in acutely isolated flexor digitorum brevis fibers had properties indistinguishable from those of muscle cells grown in tissue culture. Channel open probability in *mdx* fibers was ~2 times greater than the activity recorded from wild type fibers. The overall levels of activity in fibers, however, was roughly an order of magnitude smaller than in myoblasts or myotubes.

Experiments also compared the channels in wild type and *mdx* FDB fibers to determine whether the mechanosensitive gating mechanism is similar to the channels in

tissue cultured myoblasts and myotubes, particularly since the membrane properties of tissue cultured cells might be expected to differ from intact fibers. Experiments compared the response of channels in wild type and *mdx* fibers to suction applied to the patch electrode. Channel activity recorded from either wild type or *mdx* fibers increased with suction, however, the same amount of pressure evoked somewhat less channel activity in *mdx* fibers than in wild type fibers. In addition, although suction increased channel activity in both types of fibers during its application, channel activity recorded from *mdx* fibers did not return to control levels. This suggests that the mechanical membrane properties of normal and *mdx* fibers differ.

Gating of the stretch-inactivated channel in mdx muscle cells. Patch clamp methods were used to study the mechanosensitive gating of ion channels in post-fusion myotubes from *mdx* mice. In some recordings, channels opened only infrequently at rest. In other patches, however, channels were open continuously. During continuous recordings from cell-attached patches at a constant holding potential, channel activity increased steadily from low levels after seal formation to levels approaching unity. Strong depolarization also caused channels to open continuously. Channels having either a low or high probability of opening were distinguished by their response to pressure: applying suction to the patch electrode enhanced opening of channels with a low probability of opening, while it reduced opening of channels with a high opening probability. The dependence of channel open probability on the amount of pressure applied to the electrode for either type of mechanosensitive gating was well described by a Boltzmann relation with similar steepness. Channel inactivation in response to pressure, however, was shifted to lower pressures.

Channels with a high probability of opening frequently occurred in hot spots containing many channels, as if channel-rich membrane domains become unmasked by the loss of the energetic constraints which normally maintain channels closed at rest. A model

for mechanotransduction in muscle was proposed in which the dystrophin-based cytoskeleton organizes localized regions of membrane and couples stresses generated within the plane of the membrane to a conformational state which favours channel closure.

Block of mechanosensitive ion channels by aminoglycoside antibiotics. The mechanism of block of single mechanosensitive ion channels by aminoglycoside antibiotics was studied in acutely isolated skeletal muscle fibers from the mouse. Neomycin and other aminoglycosides reduced the amplitude of the single-channel current at negative membrane potentials in a manner consistent with a mechanism involving a fast, voltage-dependent block of the channel. In addition to the fast blocking process, neomycin, streptomycin, and dihydrostreptomycin caused the surrent to fluctuate between the open state and a subconductance level roughly one third the amplitude of the fully open state. Increasing the concentration of neomycin in the patch electrode increased the proportion of time the channel spent at the subconductance level. High concentrations of neomycin (> ~1 mM) did not reduce the amplitude of the single-channel current to a value smaller than the subconductance level. Analysis of the kinetics of the subconductance fluctuations in the presence of neomycin showed that the inverse of the mean open time depended linearly on concentration with an association rate coefficient of ~1.1 x 10^6 $M^{-1}s^{-1}$. Histograms of subconductance dwell times were best fit with two exponential components with τ_{fast} = ~100 μs and τ_{slow} = ~4 ms which were concentration-independent. Increasing the extracellular pH increased the concentration of neomycin required for both the fast block of the channel and the subconductance fluctuations. The results are interpreted in terms of a mechanism in which drug binding produces only partial occlusion of the channel conduction pathway.

Block of L-type Ca channels by aminoglycoside antibiotics. Experiments also examined the blcok of L-type Ca channels by aminoglycosides in order to obtain additional

information about the inhibitory mechanim. The activity of single L-type Ca^{2+} channels was recorded from cell-attached patches on isolated skeletal muscle fibers from the mouse. Unlike the block of mechanosensitive ion channels, aminoglycosides cause L-type channels to fluctuate between the fully open and closed state of the channel in a manner that has features suggesting the drug blocks the open channel. Other features of the blocking process, however, seem incompatible with the movement of the charged blocker to a site within the open channel. The rate of unblocking depends very little on the net charge on the aminoglycoside molecule, but more strikingly on the presence of permeant ions. The blocking rate increased as the pH was lowered in a manner consistent with the behavior of a single active drug species with a pK_a = ~7.3. The results suggest thatan interaction between permeant ion and aminoglycoside blocker at or near the channel conduction pathway.

C. List of Publications

1. Elam, T.R. and Lansman, J.B. (1993) Mechanosensitive ion channels in vascular endothelial cells. In, NATO Advanced Studies Workshop: The Role of Ion Flux in Pulmonary Vascular Control. ed., E. Kenneth Weir, Plenum Press: New York

2. Franco, A. and Lansman, J.B. (1994) Mechanosensitive ion channels in skeletal muscle from normal and dystrophic mice. J. Physiol. (in press)

3. Elam, T.R. and Lansman, J.B. (1995) The role of Mg^{2+} in the inactivation of inwardly rectifying K^+ channels in aortic endothelial cells. J. Gen. Physiol. (accepted for publication)

4. Haws, C.M., Winegar, B., and Lansman, J.B. Block of L-type Ca^{2+} channels in skeletal muscle by aminoglycoside antibiotics. Voltage, permeant ion, and pH dependence. (submitted for publication)

5. Winegar, B., Haws, C.M. and Lansman, J.B. Subconductance block of mechanosensitive Ca^{2+} channels in skeletal muscle by aminoglycoside antibiotics. (submitted for publication)

6. Franco, A. and Lansman, J.B. Mechanosensitive gating of channels in dystrophic muscle. Implications for dystrophin function. (submitted for publication)

7. Franco, A. and Lansman, J.B. Spontaneous and agonist-induced activity of acetylcholine receptor channels in developing muscle cells from normal and dystrophic mice. (submitted for publication)

Theses:

Franco-Obregón, A. (1993) A role for mechanosensitive channels during myogenesis and in muscular dystrophy. Ph.D. Thesis, University of California, San Francisco.

Elam. T.R. (1993) Gating propoerties of mechanosensitive and inwardly rectifying ion channels in vascular endothelial cells. Ph.D. Thesis, University of California, San Francisco.

D. List of Participating Scientific Personnel

Graduate students:

Alfredo Franco (Neuroscience) 1987-1993 - (Ph.D., 1993; present address, Departmento de Fisiología y Biofísica, Facultad de Medicina, Universidad de Sevilla, Sevilla, SPAIN)

Teryl R. Elam (Physiology) 1988-1993 - (Ph.D., 1993; present address, University of Washington School of Medicine)

Postdoctoral Fellows:

Bruce Winegar, Ph.D. 1989-1993 (present address, Department of Anesthesiology, School of Medicine, UCSF)

Inventions

(none)

Appendixes

1. Franco, A. and Lansman, J.B. (1994) Mechanosensitive ion channels in skeletal muscle from normal and dystrophic mice. J. Physiol. (in press)

2. Elam, T.R. and Lansman, J.B. (1995) The role of Mg^{2+} in the inactivation of inwardly rectifying K^+ channels in aortic endothelial cells. (manuscript)

3. Haws, C.M., Winegar, B., and Lansman, J.B. Block of L-type Ca^{2+} channels in skeletal muscle by aminoglycoside antibiotics. Voltage, permeant ion, and pH dependence. (manuscript)

4. Winegar, B., Haws, C.M. and Lansman, J.B. Subconductance block of mechanosensitive Ca^{2+} channels in skeletal muscle by aminoglycoside antibiotics. (manuscript)

5. Franco, A. and Lansman, J.B. Mechanosensitive gating of channels in dystrophic muscle. Implications for dystrophin function. (manuscript)

6. Franco, A. and Lansman, J.B. Spontaneous and agonist-induced activity of acetylcholine receptor channels in developing muscle cells from normal and dystrophic mice. (manuscript)